

## Roles of Metabotropic Glutamate Receptors in Brain Plasticity and Pathology

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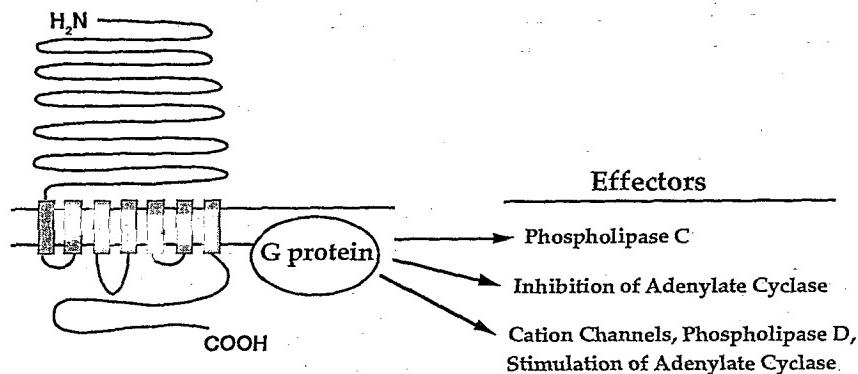
Receptors for the excitatory amino acid L-glutamate are widely expressed in both neurons and glia throughout the central nervous system. The glutamate transmitter system is the major system for the mediation of fast excitatory synaptic transmission and is also involved in neuroplasticity and higher cognitive functions (for review see ref. 1). Several subtypes of ionotropic glutamate receptors exist which are named for their selective agonists: *N*-methyl-D-aspartate (NMDA), kainate, and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA). These ionotropic receptors are composed of multiple subunits comprising an integral cation channel. In contrast, the metabotropic glutamate receptors (mGluRs), which are the focus of this paper, are a family of large monomeric receptors that exert their effects either on second messengers or ion channels via activation of GTP-binding proteins (G-proteins). The widespread distribution of both glutamate and the mGluRs suggests that glutamate may be the primary modulator of G-protein-coupled signal transduction in the central nervous system.

The mGluRs illustrate many of the emerging principles of receptor functional diversity represented in this volume. This paper provides a brief review of the diversity of the mGluRs including their structure, distribution, and the biochemical and electrophysiological consequences of their activation, but primarily focuses on their roles in brain plasticity and pathology and on the regulation of one receptor subtype in astrocytes. Studies from our laboratory and others demonstrated that agonists of the mGluRs can have either neuroprotective or neuropathological effects. These studies are reviewed, and possible explanations are discussed for the diversity of outcomes following mGluR agonist application. Finally, we present recent findings from our laboratory concerning an astrocyte model that we developed for the study of receptor regulation and function.

*Structure and signal transduction.* The members of this receptor family have a large extracellular domain (500–600 amino acids) and seven predicted transmembrane domains characteristic of G-protein-coupled receptors (FIG. 1), but have little sequence homology to other neurotransmitter receptors. The mGluRs can activate a

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variety of signal transduction mechanisms, of which the most extensively studied are the phospholipase C-mediated stimulation of phosphoinositide (PI) hydrolysis and the inhibition of adenylate cyclase activity. Hydrolysis of membrane phosphatidylinositol-4,5-bisphosphate by phospholipase C produces two second messengers: diacylglycerol, which activates protein kinase C, and inositol 1,4,5-trisphosphate ( $IP_3$ ), which elicits the release of calcium from intracellular stores. Inhibition of adenylate cyclase reduces the accumulation of the second messenger cyclic AMP. The ability of glutamate and other mGluR agonists to stimulate PI hydrolysis and inhibit cyclic AMP accumulation has been characterized in a variety of preparations including tissue slices, cultured neurons, cultured astrocytes, and transfected cell lines (see ref. 2 for review). Ample evidence also exists that these receptors can couple to G-proteins that directly gate cation channels, stimulate phospholipase D activity, and stimulate adenylate cyclase activity. To date, seven subtypes (mGluR1-7)<sup>3-9</sup> have been identified, some of which are present in more than one splice variant.



**FIGURE 1:** Schematic illustration demonstrating the salient features of the mGluRs which include a large extracellular domain and seven predicted membrane spanning regions. The receptors are coupled via G-proteins to a variety of effectors including phospholipase C, adenylate cyclase, and cation channels.

form.<sup>5,10,11</sup> These subtypes can be organized into three subfamilies<sup>12</sup> based upon their sequence homology, the effector system to which they couple when artificially expressed in CHO or BHK cells, and the agonist selectivity for activation of the receptor (TABLE 1).

*Electrophysiological effects.* The electrophysiological consequences of mGluR activation have been extensively studied and both pre- and postsynaptic effects have been identified. These studies were greatly facilitated with the identification of the selective agonist 1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD),\* a conformationally restricted analog of glutamate with selectivity for metabotropic

\*Most early studies with *trans*-ACPD used the racemic *trans*-(±)-ACPD composed of a mixture of the 1S,3R-ACPD and 1R,3S-ACPD enantiomers. More recently the separate enantiomers have also become commercially available and the more active 1S,3R-ACPD is frequently used.

receptors.<sup>16</sup> Activation of mGluRs results in both excitatory and inhibitory actions (for reviews see refs. 2 and 17). For example, in the hippocampus, *trans*-ACPD can produce depolarization and reduction of the after-hyperpolarization via a blockade of a calcium-activated potassium current. It has also been shown to block accommodation of cell firing, increase the amplitude of population spikes, induce generation of multiple spikes, decrease paired-pulse inhibition, and decrease evoked inhibitory postsynaptic potentials. Inhibitory actions of *trans*-ACPD in the hippocampus include reduction of the field excitatory postsynaptic potential via a presynaptic action.

**Distribution.** The mGluRs are expressed in neuronal and glial populations throughout the brain (see ref. 1 for review). These receptor subtypes show distinct patterns of distribution with differential expression both regionally and between cell types within a region. Each subtype has a unique pattern of expression although these patterns are sometimes overlapping. Some subtypes are widely expressed; mGluR3, for example, is prominently expressed in neurons in the cerebral cortex, thalamus, caudate putamen, and dentate gyrus and in glial cells throughout the brain.<sup>12</sup> The expression of other subtypes is quite restricted. For example, mGluR6

TABLE I. Subgroups of the Metabotropic Glutamate Receptors<sup>a</sup>

Sub-group	Gene	Effector System	Agonist Selectivity	Ref.
I	mGluR1 mGluR5	Stimulate phospholipase C	QA > Glu ≥ Ibo > <i>t</i> -ACPD ≫ AP4	3, 4, 13, 14 6
II	mGluR2 mGluR3	Inhibit adenylyl cyclase	Glu ≥ <i>t</i> -ACPD > Ibo ≫ QA ≫ AP4	5 5, 12
III	mGluR4 mGluR6 mGluR7	Inhibit adenylyl cyclase	AP4 > Glu > SOP > ACPD > QA > Ibo AP4 > SOP > Glu ≫ ACPD > QA/Ibo AP4 = SOP > Glu > QA/ACPD	5, 12, 15 7 8, 9

<sup>a</sup>The seven metabotropic glutamate receptors can be classified into three subgroups based upon similarities in their sequence, effector coupling systems, and agonist selectivity.<sup>12</sup>

displays the most restricted expression of all the mGluRs, showing appreciable expression only in the inner nuclear layer of the retina, the region containing the ON-bipolar cells.<sup>7</sup> In addition to the regional variation in receptor expression it is interesting to note the degree of differential expression within a region. In the cerebellum for example, there is prominent expression of three separate subtypes: mGluR1 in Purkinje cells, mGluR2 in Golgi cells, and mGluR4 in granule cells. Such precise segregation of receptors implies an important role for the subtypes in functional specialization.

#### INVOLVEMENT OF METABOTROPIC GLUTAMATE RECEPTORS IN PLASTICITY

A substantial body of evidence now exists indicating that mGluRs have important roles in development and plasticity. For example, the developmental peak of excitatory amino acid-stimulated PI hydrolysis occurs between 6 and 12 days of age in neonatal rats and exhibits a high correlation with periods of intense synaptogenesis.<sup>18,19</sup> More direct evidence for a role of mGluRs in plasticity is provided by studies of long-term potentiation (LTP) and long-term depression (LTD). In the CA1 region of hippocampal slices, bath application of *trans*-ACPD can produce a form of LTP

even without concomitant tetanic stimulation<sup>20</sup> and application of *trans*-ACPD in conjunction with tetanic stimulation potentiates the amount of LTP produced.<sup>21</sup> Most recently, it has been shown that the newly characterized metabotropic receptor antagonist  $\alpha$ -methyl-4-carboxyphenylglycine (MCPG) can block the induction of LTP without affecting baseline synaptic transmission or previously established LTP.<sup>22,23</sup> Similar studies have indicated a role for mGluRs in LTD in both CA1 and in the parallel fiber-Purkinje cell synapse in the cerebellum.<sup>24</sup>

#### NEUROPROTECTIVE ACTIONS OF mGluR AGONISTS

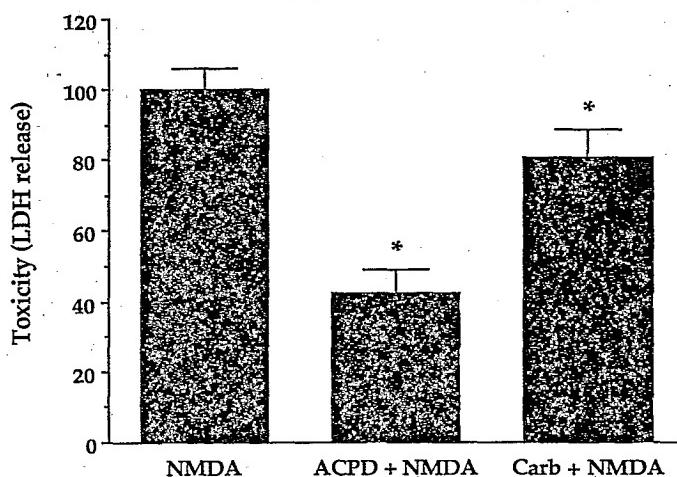
Involvement of mGluRs in pathology was initially suggested when enhancements of excitatory amino acid-stimulated PI hydrolysis were demonstrated in *ex vivo* brain slice preparations following kindling,<sup>25</sup> hippocampal lesions,<sup>26</sup> and transient global ischemia.<sup>27</sup> These studies provided intriguing correlations between pathological conditions and mGluR activity, but could not address whether this increased receptor activity was part of the pathological cascade or part of a compensatory, possibly protective response to the injury. Additionally, such brain slice studies could not address the relative contributions of neurons and glia to these enhancements in PI hydrolysis. In the case of the ionotropic glutamate receptors, it has been well documented that overactivation of these receptors can produce pathological excitotoxicity through a process that involves calcium influx.<sup>28</sup> Several lines of evidence indicate that excitotoxicity is involved in the pathogenesis of trauma and ischemia as well as some neurodegenerative diseases.<sup>29,30</sup>

The involvement of mGluRs in excitotoxicity was first directly addressed in our laboratory when Koh, *et al.*<sup>31,32</sup> quantified the effects of *trans*-ACPD in a mixed neuron-glial murine cortical culture model of excitotoxicity. As with the ionotropic receptors, activation of mGluRs coupled to PI hydrolysis also increases the cytoplasmic calcium concentration, leading to the initial hypothesis that mGluR agonists would also contribute to excitotoxicity. However, application of *trans*-ACPD did not produce neurotoxicity even when applied at concentrations as high as 1 mM for 24 h.<sup>32</sup> In contrast, extensive neuronal degeneration was produced following a 5-min exposure to 500  $\mu$ M of the ionotropic receptor agonist NMDA (FIG. 2), as had been previously demonstrated. Modulation of NMDA toxicity by *trans*-ACPD was next examined with simultaneous exposure to the two agonists. Surprisingly, the presence of 100  $\mu$ M *trans*-ACPD during the exposure of the cultures to NMDA markedly attenuated the NMDA-induced excitotoxicity (FIG. 2). This protective effect was not limited to glutamate receptor activation because the cholinergic receptor agonist carbachol, another activator of PI hydrolysis, had a similar but more modest protective effect, suggesting that production of excitotoxicity can be influenced by interaction between multiple receptor subtypes and transmitter systems.

The demonstration that mGluR agonists can have neuroprotective effects has now been replicated in both cortical and cerebellar cultures,<sup>33,34</sup> and other neuroprotective actions have been demonstrated both *in vitro* and *in vivo*. In rat hippocampal slices subjected to *in vitro* hypoxia, the presence of 1S,3R-ACPD during hypoxia was neuroprotective as measured by an enhancement in the posthypoxic recovery of field excitatory postsynaptic potentials.<sup>35</sup> Similarly, in an *in vivo* murine ischemia model, *trans*-ACPD administered intraperitoneally immediately after middle cerebral artery occlusion reduced the size of the infarct volume.<sup>36</sup> Finally, preinjection of 1S,3R-ACPD into the retina of adult rats has been shown to reduce the neurotoxicity produced by a subsequent injection of NMDA.<sup>37</sup>

Inasmuch as glutamate is necessary for normal physiological functioning, but

overexcitation by this transmitter can lead to pathological conditions of excitotoxicity, it seems likely that specific mechanisms must exist to control this balance between normal functioning and pathology. Based on the results reviewed above, it is intriguing to speculate that activation of mGluRs may be one of these mechanisms. However, the scenario appears to be quite complex as several *in vivo* studies have also shown that activation of these receptors can have neurotoxic consequences rather than neuroprotective effects.



**FIGURE 2.** Attenuation of NMDA toxicity in murine cortical cultures (15–16 days *in vitro*). Release into the medium of an intracellular enzyme, lactate dehydrogenase (LDH), was quantified as an indicator of cell death. LDH release was measured 24 h after a 5-min exposure to NMDA alone or in the presence of 100  $\mu$ M *trans*-( $\pm$ )-ACPD (ACPD) or 500  $\mu$ M carbachol (Carb). Data shown are mean  $\pm$  SEM of three experiments performed in quadruplicate. LDH values were scaled to the mean value in the NMDA-treated cultures after subtraction of the mean value in sister cultures exposed to sham washes. Asterisk denotes differences from the control ( $p < 0.05$ , two-tail *t* test with Bonferroni correction for two comparisons).

#### NEUROPATHOLOGICAL ACTIONS OF mGluR ACTIVATION

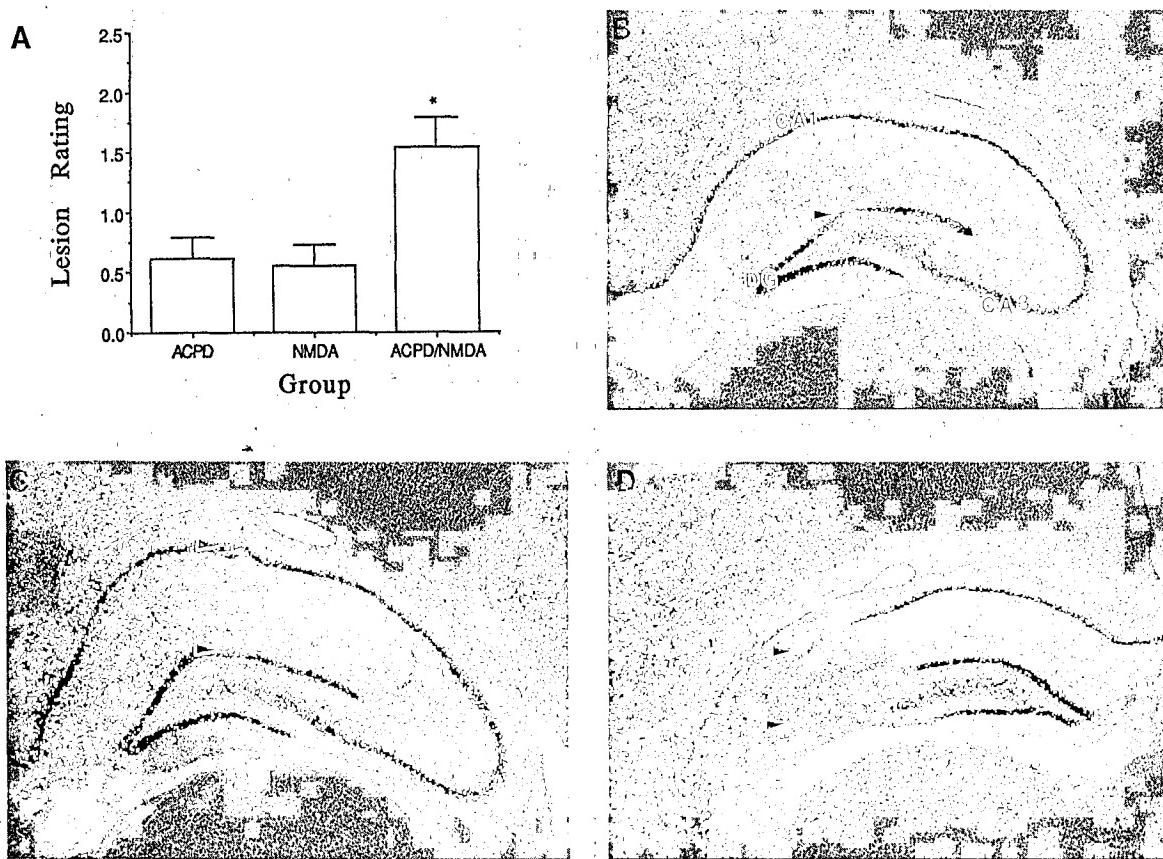
Several previous studies demonstrated both behavioral disturbances and neuro-anatomical pathology following administration of *trans*-ACPD. Intrahippocampal injection of 1S,3R-ACPD into adult rats produced seizure activity characterized by akinesia, wet-dog shakes, rearing, limbic seizures, and hyperactivity.<sup>38,39</sup> In addition to seizures, 1S,3R-ACPD also produced neuropathology including loss of CA1 and CA4 pyramidal neurons and dentate gyrus granule neurons 6–7 days after injection.<sup>39</sup> Striatal injections in neonatal rats produced little pathology but did potentiate NMDA-induced injury as measured by reduction in brain weight 5 days after injection.<sup>40</sup> However, injection of higher doses of *trans*-ACPD alone into the hippocampus or striatum of neonates produced loss of brain weight and signs of neuropathology including swollen perinuclear cytoplasm, extracellular debris, and shrunken nuclei that were observed at 4 h but not 5 days following injection.<sup>41</sup>

We recently began studies to evaluate the effects of mGluR agonists in the hippocampus *in vivo*, focusing on the effects of lower concentrations of agonists, interaction with NMDA toxicity, and assessment of long-term anatomical outcome.<sup>42</sup> Intrahippocampal administration of 1S,3R-ACPD was found to produce dose-dependent damage when brains of young adult rats were examined 14 days after injection. Interaction between the ionotropic and mGluR receptor pathways was then examined using combinations of minimally toxic doses of 1S,3R-ACPD and NMDA to provide an *in vivo* parallel to the previous *in vitro* studies from our laboratory. Intrahippocampal injection of 1  $\mu$ L of 10 mM 1S,3R-ACPD produced only slight neural damage to the dorsal blade of the dentate gyrus (FIG. 3B). Administration of 1  $\mu$ L of 100 mM NMDA also produced limited degeneration in the dentate gyrus as well as in CA1. However, the combination of 1S,3R-ACPD and NMDA produced a much larger lesion, involving the lateral dentate gyrus and CA1 to CA3. (FIG. 3D). Although in contrast to the protective effects that have been demonstrated in other preparations, this enhancement of toxicity is consistent with the initial hypothesis that activation of mGluRs should contribute to excitotoxicity by elevating levels of intracellular calcium. Activation of an mGluR coupled to PI hydrolysis could increase calcium levels either by IP<sub>3</sub>-mediated release of calcium from intracellular stores, or by diacylglycerol activation of protein kinase C and a subsequent facilitation of calcium current through NMDA receptors.<sup>43</sup>

#### PLASTICITY VERSUS PATHOLOGY

What are the reasons why both neuroprotective and neurotoxic effects have been reported following application of metabotropic agonists? One important consideration involves the differing methodological limitations of various preparations. Results from *in vitro* preparations must be interpreted with caution because these are artificially simplified systems, often lacking the full complement of cell types and the proper connectivity found *in vivo*. Although *in vivo* preparations are obviously desirable to examine the roles of mGluRs in intact systems, they also have inherent limitations. For example, the use of local injections often results in the introduction of extremely high local concentrations of agonists. Concentrations of 1S,3R-ACPD in the hundreds of millimolar have been used for intrahippocampal injections,<sup>38,39</sup> concentrations at which the selectivity of the compound for mGluRs is markedly reduced.<sup>16</sup> In fact, recent work from our laboratory suggests that there is pharmacological overlap between inhibitors of glutamate transport and mGluR agonists,<sup>44</sup> and high concentrations of *trans*-( $\pm$ )-ACPD have previously been reported to inhibit glutamate uptake.<sup>45</sup> The resulting increase in extracellular glutamate would produce activation of both ionotropic receptors and mGluRs. In contrast, most of the methods of application that have yielded protective results with *trans*-ACPD (application of the agonist in culture medium,<sup>31,33</sup> in hippocampal slice bathing medium,<sup>35</sup> with intraperitoneal injection,<sup>36</sup> or intraocular injection<sup>37</sup>) allow greater diffusion of the agonist than what is probably achieved following intracerebral injection. Thus, crossover between uptake inhibition and receptor activation may at least partially account for the toxicity demonstrated in some preparations. Clarification of this issue will require development of increasingly selective pharmacological tools with less cross-reactivity between glutamate transport and mGluRs.

Another aspect which may contribute to the variation in the effects observed with mGluR agonists is differential expression of the mGluR subtypes in the various preparations that have been used. The ability of *trans*-ACPD to produce neurotoxic effects appears to be dependent upon a number of variables including age, brain



**FIGURE 3.** Excitotoxic interaction between 1S,3R-ACPD and NMDA following intrahippocampal injection. (A) Animals received unilateral intrahippocampal injections of either 1S,3R-ACPD (1  $\mu$ L of 10 mM;  $n = 9$ ) or NMDA (1  $\mu$ L of 100 mM;  $n = 13$ ). Both agonists together were administered to the contralateral hippocampus of each animal. Lesion size 14 days following injections was rated on a 3-point scale: 0 = no damage, 1 = mild, 2 = moderate, 3 = severe damage, and is presented as mean  $\pm$  SEM. Asterisk denotes difference from NMDA alone ( $p < 0.05$ ). (B) Injection of 1  $\mu$ L of 10 mM of 1S,3R-ACPD by itself produced only minimal localized damage to the dorsal blade of the dentate gyrus (DG, arrow). (C) Injection of 1  $\mu$ L of 100 mM NMDA produced minor damage to the CA1 and DG (arrows) surrounding the cannula tract and the site of injection. (D) Administration of 1S,3R-ACPD + NMDA increased the neurotoxic activity with extensive neuronal loss in the CA2 and CA3, the lateral aspect of the dorsal blade of the DG, and part of CA1 (arrows).

region, and strain of animal used. These types of differences could be due to differences in the amounts and ratios of receptor subtype expression. The mGluR subtypes can couple to a variety of signal transduction mechanisms, producing either excitatory or inhibitory effects. Thus, whether the net effect of an mGluR agonist is neuroprotective or neuropathological might depend upon the relative expression of specific metabotropic receptor subtypes coupled either to excitatory or inhibitory transduction mechanisms. As more selective pharmacological tools become available, progress can be made in evaluating the neurotoxic and neuroprotective effects of specific receptor subtypes. Further, development of experimental means with which to up-regulate or down-regulate specific mGluR subtypes may aid in examining the functional roles of these receptors.

#### REGULATION OF METABOTROPIC RECEPTORS—AN ASTROCYTE MODEL SYSTEM

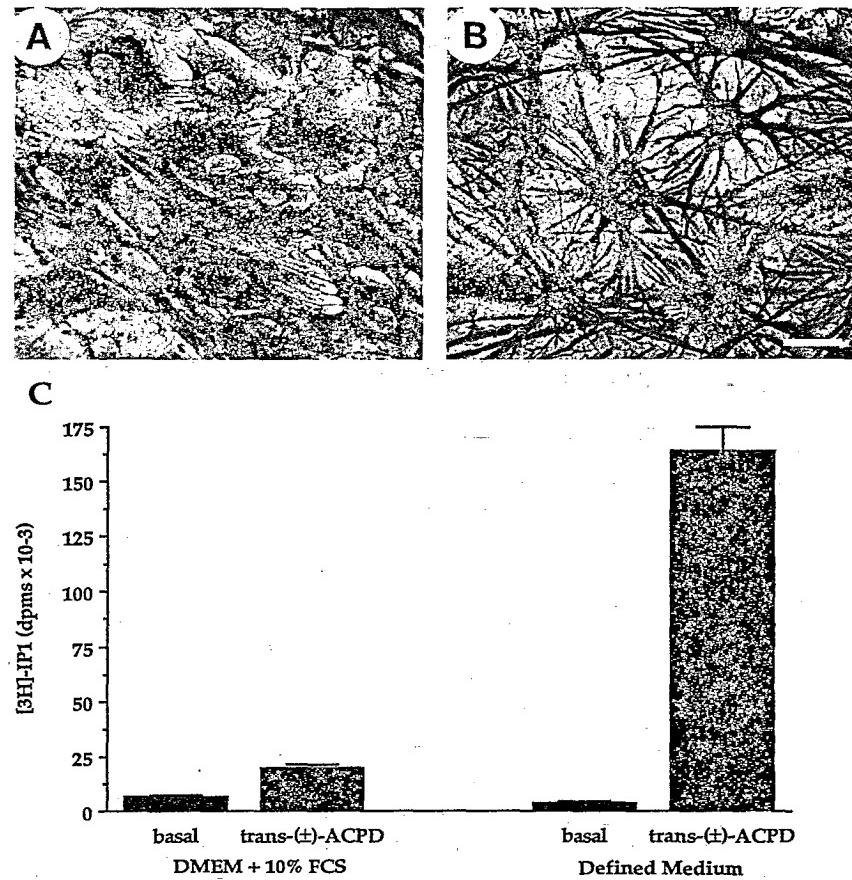
The mGluRs of astrocytes may play a role in determining the outcome following administration of mGluR agonists *in vivo*. Astrocytes are a major component of glutamatergic pathways; their processes envelop glutamatergic synapses, and they have important roles in maintaining the balance between normal excitatory transmission and excitotoxicity, for example, through sodium-dependent high-affinity transport of glutamate.<sup>46,47</sup> Because activation of the mGluRs can have either neuroprotective or neurotoxic effects, we became interested in exploring the factors that regulate mGluR expression and in identifying more selective pharmacological tools with which to dissociate mGluR activation and transport inhibition. Astrocyte culture provides a system readily accessible to the study of transport pharmacology as well as mGluR signal transduction and receptor expression. Although some subtypes of mGluRs were known to be expressed in astrocytes, their function had not been previously determined. To approach an integrated functional understanding of these receptors the first step was to define a suitable *in vitro* model for their study in astrocytes.

##### *Use of Serum-free Defined Medium*

We began studies of metabotropic signal transduction and mGluR expression by analyzing *trans*-ACPD stimulation of PI hydrolysis using astrocytes cultured with conventional techniques. Primary glial cultures were prepared from neocortices of neonatal rat pups and purified by shaking as previously described.<sup>48</sup> After 7–8 days, secondary astrocyte cultures were established by trypsinizing and subplating into Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS).

Although widely used, this method of culture was unsatisfactory for our studies for several reasons. First, the morphology of astrocytes grown under these conditions is not representative of the morphology of the majority of astrocytes *in vivo*. Astrocytes grown in the FCS-supplemented medium were flat and polygonal in shape, having few processes (FIG. 4A), unlike the branching and stellate morphology of astrocytes *in vivo*. Second, the use of serum as a media supplement is a poor physiological model because exposure of brain cells to serum is normally limited *in vivo* by the blood-brain barrier. Third, culturing in serum-containing media has the disadvantage that serum contains a complex, undefined, and variable mixture of

Growth cones of the DG, and part of CA1 (arrows)



**FIGURE 4.** Glial fibrillary acid protein (GFAP) immunostaining of secondary astrocyte cultures maintained in either DMEM + 10% FCS (A) or in the serum-free defined medium (B). GFAP immunostaining was performed using a polyclonal rabbit primary antibody (1:2000 dilution; Dako, Carpinteria, CA) and avidin-biotin conjugated secondary antibody (ABC kit, Vector, Burlingame, CA) followed by visualization with diaminobenzidine. (C) Demonstration of the maximal stimulation of PI hydrolysis by *trans*-ACPD in these two types of cultures. Following a 24-h labeling period with [<sup>3</sup>H]-*myo*-inositol (2  $\mu$ Ci/well), culture medium was aspirated and the cultures washed and preincubated for 20 min in buffer containing 10 mM LiCl (to inhibit *myo*-inositol-1-phosphatase), 116 mM NaCl, 26.2 mM NaHCO<sub>3</sub>, 1 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.5 mM KCl, 1.5 mM MgSO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub>, and 20 mM glucose. Cultures were then stimulated with 500  $\mu$ M *trans*-( $\pm$ )-ACPD for 60 min, followed by isolation of [<sup>3</sup>H]-IP<sub>1</sub> with anion exchange chromatography and liquid scintillation counting essentially as described.<sup>55</sup> Values are mean  $\pm$  SEM from five experiments performed in triplicate.

growth-promoting and -inhibiting components; this was a particular problem for these studies because we were interested in studying signal transduction and receptor expression in the context of neuroglial interaction, and thus required a model in which we had precise control over the concentrations of individual growth factors or cytokines. For these reasons we modified our culture techniques to the use of a serum-free chemically defined medium.<sup>49</sup> This method employed the same basal medium, DMEM, but rather than supplementing with FCS it was supplemented with eight defined components: transferrin (50 µg/mL), D-Biotin (10 ng/mL), selenium (5.2 ng/mL), fibronectin (1.5 µg/mL), heparin sulfate (0.5 µg/mL), epidermal growth factor (10 ng/mL), fibroblast growth factor (5 ng/mL), and insulin (5 µg/mL); a modification of the G-5 medium of Michler-Stuke *et al.*<sup>50</sup> When cultured under these conditions astrocytes had a highly branched, stellate shape (FIG. 4B), more similar to the morphology of astrocytes *in vivo*.

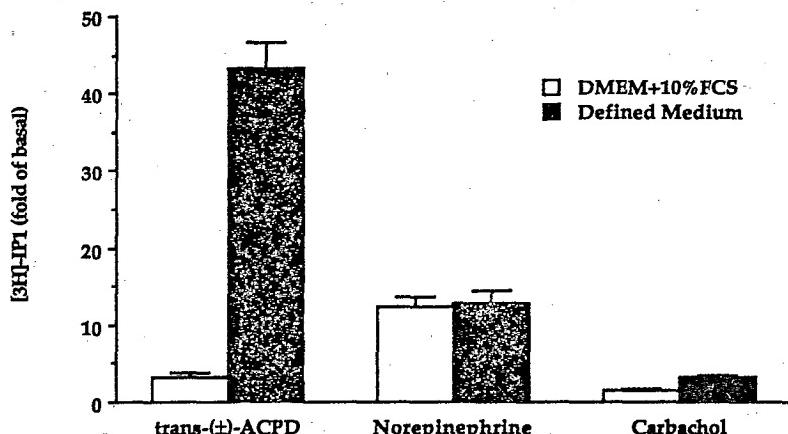
#### *Robust Stimulation of Phosphoinositide Hydrolysis*

In addition to the morphological changes, this manipulation of the growth conditions produced a radical alteration in the signal transduction properties of these cells.<sup>49</sup> When astrocytes were grown in secondary culture with FCS-supplemented DMEM, application of a maximally effective concentration of *trans*-ACPD (500 µM, 60 min) produced an accumulation of inositol monophosphate (IP<sub>1</sub>), which was approximately threefold that of unstimulated cultures ( $3.2 \pm 0.5$  fold of basal,  $n = 5$ ; FIG. 4C). However, in sister cultures maintained in secondary culture in the defined medium, *trans*-ACPD stimulated IP<sub>1</sub> accumulation more than 40-fold ( $43.2 \pm 3.6$  fold of basal,  $n = 5$ ; FIG. 4C). The ability of two other mGluR agonists, quisqualate and glutamate itself, to stimulate PI hydrolysis was similarly enhanced (not shown). This exciting finding indicates a dynamic capability for astrocytes to respond to glutamate and suggests that previous studies using astrocytes cultured with serum-containing media had underestimated this potential.

Subsequently we examined the mechanism for this dramatic change in the PI response to determine whether this alteration represented a nonspecific sensitization of PI hydrolysis or whether the alteration was more selective to some component of an mGluR signaling pathway. When stimulation of PI hydrolysis by agonists of other transmitter systems was evaluated, a similar marked enhancement in PI hydrolysis was not observed. The accumulation of IP<sub>1</sub> stimulated by 500 µM norepinephrine was the same in DMEM + 10% FCS cultures ( $12.3 \pm 1.3$  fold of basal,  $n = 3$ , FIG. 5) as in defined medium cultures ( $12.6 \pm 1.8$  fold of basal,  $n = 3$ ), whereas stimulation by the muscarinic receptor agonist carbachol (500 µM) was only moderately increased in defined medium cultures ( $3.1 \pm 0.3$  fold of basal,  $n = 3$ , FIG. 5) compared to DMEM + 10% FCS cultures ( $1.5 \pm 0.2$  fold of basal,  $n = 3$ ). Because the dramatic enhancement in the stimulation of PI hydrolysis appeared to be selective for glutamate agonists, we hypothesized that exposure to the defined medium components had altered some component of a glutamatergic signal transduction system.

#### *Regulation of mGluR Expression*

We next evaluated the expression of the mGluRs themselves. Of the seven mGluR subtypes that have been characterized, two of these—mGluR1 and mGluR5—have been shown to be able to couple to PI hydrolysis.<sup>6,14</sup> Astrocytes were maintained



**FIGURE 5.** Stimulation by the indicated agonists (500  $\mu$ M, 60 min) of [<sup>3</sup>H]-IP<sub>1</sub> accumulation in astrocytes prelabeled with [<sup>3</sup>H]-myo-inositol. Values are mean  $\pm$  SEM from five experiments with trans-( $\pm$ )-ACPD performed in triplicate and three experiments performed in triplicate with norepinephrine and carbachol. Basal (unstimulated) levels of [<sup>3</sup>H]-IP<sub>1</sub> accumulation were  $6,830 \pm 820$  dpm/well in DMEM + 10% FCS cultures and  $4,010 \pm 300$  dpm/well in defined medium cultures.

for 4–5 days in secondary culture in DMEM supplemented with either 10% FCS or the defined medium supplements. Cultures were then harvested and the membranes prepared for Western immunoblotting against antibodies specific for either mGluR1 $\alpha$  or mGluR5. Using an antibody prepared and characterized by Martin *et al.*,<sup>51</sup> we found no mGluR1 $\alpha$  signal in membranes prepared from astrocytes cultured under either condition. This was not surprising as *in situ* hybridization and immunocytochemistry of brain slices have shown that mGluR1 is restricted to neuronal elements.<sup>51,52</sup>

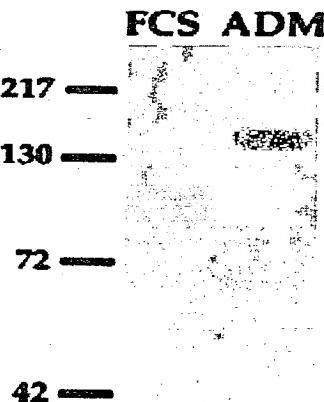
However, mGluR5 does appear to be expressed in astrocytes. An affinity-purified antibody selective for mGluR5 was generated as described<sup>53</sup> using a synthetic peptide corresponding to the C-terminal 13 amino acids of the predicted polypeptide sequence of the mGluR5 cDNA.<sup>6</sup> The peptide was conjugated to thyroglobulin using glutaraldehyde, antisera prepared, and antibodies affinity-purified using standard techniques.<sup>54</sup> The anti-mGluR5 antibody should recognize both known splice variants mGluR5 $\alpha$  and mGluR5 $\beta$ ,<sup>11</sup> because the variants have identical C-terminal sequences, but the antibody does not recognize mGluR1 $\alpha$  (not shown). Although there was little or no mGluR5 present in membranes prepared from DMEM + 10% FCS cultures, the antibody recognized a strong band at approximately 145 kDa in membranes from defined medium cultures (FIG. 6). Thus, a large induction in the expression of mGluR5 corresponds with the increased ability of mGluR agonists to stimulate PI hydrolysis. The results obtained with this relatively simple manipulation of the culture medium indicate that expression of the receptor subtypes can be plastic and that differential plasticity exists even between subtypes coupled to the same transduction mechanism.

#### SUMMARY

In summary, the mGluRs are a large family of receptor subtypes with diverse properties in terms of transduction coupling, pharmacology, and anatomical distribu-

tion. Many divergent studies have demonstrated that activation of these receptors can result in either neuroprotection or neuropathology. We hypothesized that the mGluRs of astrocytes may have a role in determining the response following administration of mGluR agonists *in vivo*, and we have defined a suitable *in vitro* model for the study of these receptors. The experimental plasticity demonstrated in the astrocyte culture model may represent a more general principle that conditions in the microenvironment may differentially alter mGluR subtype expression as part of development, functional specialization, or pathology. This astrocyte model of receptor regulation provides a system suitable for studying the effects of specific growth factors, neurotrophins, cytokines, and other substances released by neurons and glia that may act in both autocrine and paracrine fashions. Alteration in the ratios of receptors by such variables could then modify future signaling properties and neuroglial interactions, a form of conditioning of the astrocytic response that would alter the physiological output following glutamate release.

One measure of the value of this model will be its usefulness in stimulating the generation of hypotheses that can be tested *in vivo*. For example, the morphology of the astrocytes when cultured in the defined medium has similarities to the morphology of astrocytes undergoing reactive gliosis in pathological states. It is also interesting to note that treatments that have been reported to increase excitatory amino



**FIGURE 6.** mGluR5 Western blotting of astrocyte membranes. Standard fractionation techniques were used to prepare crude membrane fractions from astrocytes cultured in either DMEM supplemented with 10% fetal calf serum (FCS) or cultured in the astrocyte defined medium (ADM). For electrophoresis, membranes were dissolved in sample buffer containing 20 mM dithiotreitol and subjected to SDS-PAGE in 7.5% gels. Separated proteins were transferred to Immobilon P membranes (Millipore, Bedford, MA) and incubated in TTBS (50 mM Tris HCl, 154 mM NaCl, 0.1% Tween-20, pH 7.5) containing 2.5% nonfat dry milk for 15 min, then overnight in the same buffer together with antibody (1:2500) and 0.1% sodium azide. After several washes in TTBS, the membranes were incubated in TTBS/2.5% milk containing goat anti-rabbit coupled to horseradish peroxidase (1:2000, GAR-HRP; Fisher, Pittsburgh, PA) for 2 h. After several washes in TTBS immunoactive bands were visualized using enhanced chemiluminescence (ECL reagent, Amersham, Arlington Heights, IL). The comparison between the two culture types was repeated four times using different astrocyte preparations. A representative blot is shown.

acid-stimulated PI hydrolysis in *ex vivo* brain slices (lesions,<sup>26</sup> ischemia,<sup>27</sup> and kindling<sup>25</sup>) are accompanied by reactive gliosis. Those findings combined with the present *in vitro* results lead us to speculate that mGluR5 expression may also be altered *in vivo* during reactive gliosis. If so, it will be important to examine the functional consequences of such a change with regard to the astrocytic response to injury and maintaining the balance between excitatory transmission and excitotoxicity.

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